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Androgen Receptor-Mediated Antagonism of Estrogen-Dependent Low Density Lipoprotein Receptor Transcription in Cultured Hepatocytes

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ABSTRACT

Postmenopausal women receiving hormone replacement therapy have a lower risk of coronary heart disease than women who do not receive hormone treatment. Multiple mechanisms are likely to underlie estrogen's cardioprotective action, including lowering of plasma low density lipoprotein (LDL) cholesterol. Using an *in vitro* system exhibiting normal regulation of LDL receptor (LDLR) gene transcription, we show that 17β-estradiol activates the LDLR promoter in transiently transfected HepG2 cells. LDLR activation by estrogen in HepG2 cells is dependent on the presence of exogenous estrogen receptor, and the estrogen-responsive region of the LDLR promoter colocalizes with the sterol response element previously identified. The estrogen response is concentration dependent, saturable, and sensitive to antagonism by estrogen receptor antagonists. Further, we show that compounds with androgen receptor agonist activity attenuate the estrogen-induced up-regulation of LDLR in our model system. Progestins with androgen receptor agonist activity, such as medroxyprogesterone acetate, also suppress estrogen's effects on LDLR expression through their androgenic properties. Characterization of the interplay between these hormone receptors on the LDLR *in vitro* system may allow a better understanding of the actions of sex steroids on LDLR gene expression and their roles in cardiovascular disease. (*Endocrinology* **138**: 3779–3786, 1997)

CARDIOVASCULAR disease is the leading cause of death for women in Western society. The cardiovascular disease risk for premenopausal women is lower than that for men of comparable age, but the risk for postmenopausal women meets and possibly exceeds the risk for men in the same age group (1). The increased risk appears to be due to decreasing estrogen levels, and hormone replacement therapy reduces the risk of cardiovascular disease in postmenopausal women. Estrogens administered to female monkeys reduced atherosclerotic plaque development (2), and estrogens administered to postmenopausal women increase low density lipoprotein (LDL) clearance (3), lower plasma LDL cholesterol levels (3–6), reduce atherosclerosis (7, 8), and reduce the risk of cardiovascular disease (9–16). The mechanisms underlying these beneficial effects of hormone replacement therapies are the subject of intense study. Proposed mechanisms by which estrogen mediates cardioprotection include alteration of the vascular wall, hemostasis, and plasma lipids. A clearer understanding of the mechanisms by which estrogens affect cardiovascular disease may allow improved hormone replacement therapy.

One effect of estrogen is to lower plasma LDL cholesterol, a known cardiovascular risk factor (1). Plasma levels of LDL cholesterol are regulated in part by clearance of LDL by hepatic LDL receptor (LDLR), a cell surface receptor that binds and internalizes LDL (17). The more LDLR present in the liver, the greater the rate of hepatic LDL clearance, leading to a concomitant reduction in plasma LDL cholesterol. The up-regulation of LDLR transcription by inhibitors of hydroxymethylglutaryl-coenzyme A reductase leads to a lowering of plasma LDL cholesterol, and is a primary mechanism of action for these drugs (18). Binding sites in the LDLR promoter recognized by Sp1 and sterol response element-binding protein-1 (SREBP-1) and SREBP-2 are required to observe the repression of LDLR transcription by sterols and the activation of LDLR transcription by hydroxymethylglutaryl-coenzyme A reductase inhibitors (19, 20). A proposed mechanism for the activation of LDLR transcription in response to lowered intracellular cholesterol levels is enhanced proteolytic cleavage of SREBP to a transcriptionally active form (21, 22). Although the increased LDL-binding activity in response to estrogen has been reported *in situ* (23), in animals (24), and in women (3), the mechanisms by which estrogens regulate LDLR expression have remained unclear.

Estrogens administered during the course of postmenopausal hormone replacement therapy are often accompanied by progestins to reduce the risk of endometrial cancer associated with unopposed estrogen administration. The effect of progestins on lipid profiles and cardiovascular risk in women remains unclear (13–16). Some progestins coadministered with estrogen do not significantly affect lipid profiles or the development of atherosclerosis (25, 26). However, progestins such as medroxyprogesterone acetate (MPA) attenuate the favorable lipid profile induced by estrogen (16, 27–29). Progestins differ in their selectivity for progesterone receptor (PR), and cross-reactivity with related steroid hormone receptors may be involved in the lipid-altering effects of progestins. For example, the adverse effect of MPA and certain other progestins on lipid profiles may be due to the

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androgenic activity of these compounds, rather than to activation of the PR (30–32).

To study the mechanism of LDL lowering by estrogen, we transiently transfected LDLR promoter-reporter plasmids into HepG2 cells, a human hepatocarcinoma cell line. We report a significant up-regulation of LDLR promoter-driven transcription by estrogen in an estrogen receptor (ER)-dependent manner. LDLR activation appeared specific to ER among the sex steroid receptors. Androgen receptor (AR) agonists opposed the up-regulation of LDLR by ER, lending support to the hypothesis that progestins that have androgenic activity alter lipid profiles through the AR. The mechanisms by which progestins or androgens may affect plasma lipid levels or cardiovascular risk in conjunction with estrogen replacement therapy remain unclear. We describe assays that may provide convenient models for the modulation of LDLR expression by estrogens and androgenic compounds and may help in the design of hormone replacement therapy.

Materials and Methods

Reagents

All reporter vectors were constructed with promoter elements driving the expression of the firefly luciferase gene (33). The LDLR promoter from −600 to 53 (34) fused to the coding sequence of the firefly luciferase gene in a pBLCAT-derived vector (35) was provided by Sharon Dana, Ligand Pharmaceuticals (San Diego, CA). The minimal LDLR promoter was constructed by multimerization of the Sp1- and SREBP-binding sites from the LDLR promoter region (Fig. 2A, Ref. 19) placed upstream of the minimal adenovirus E1B TATA promoter (36). The ERE-tk-LUC reporter contains the consensus estrogen response element (ERE) sequence AGGTCACAGTGACCT in a pBLCAT-derived vector placed upstream of the herpes simplex virus-thymidine kinase minimal promoter from −105 to 51 driving luciferase expression. The expression vectors for human ER, PR, and AR contained full-length coding sequence driven by the Rous sarcoma virus (RSV) long terminal repeat in a pBR322-derived vector (37). RSV-β-galactosidase plasmid was included for normalization of luciferase activity by β-galactosidase activity in all transfection data, and pGEM plasmid was used to maintain a constant quantity of DNA in transfections. ER DNA-binding mutant expression vectors were obtained originally from S. Adler (38) (Washington University, St. Louis, MO) and used as modified by B. Allegretto (39) (Ligand Pharmaceuticals). ER-mut1 contains a single mutation changing zinc finger cysteine 221 to glycine, and ER-mut2 contains both the 221 mutation and mutation of cysteine 185 to serine.

17β-Estradiol and progesterone were purchased from Aldrich Chemical Co. (Milwaukee, WI). 25-Hydroxycholesterol, MPA, tamoxifen, and clomiphene were purchased from Sigma Chemical Co. (St. Louis, MO). R-1881 was obtained from New England Nuclear Products (DuPont, Wilmington, DE). Keoxifen was provided by Ligand Medicinal Chemistry (San Diego, CA).

Cell culture and transfections

HepG2 cells were cultured in monolayer in Eagle's MEM containing 10% (vol/vol) FBS (HyClone Laboratories, Logan, UT) and supplemented with 200 mM L-glutamine (BioWhittaker, Walkersville, MD) and 55 μg/ml gentamicin (BioWhittaker). Subconfluent HepG2 cells were passed at 3-day intervals to maintain reproducible transfection efficiency and were plated in gelatin-coated 96-well plates (Costar, Cambridge, MA) 24 h before transfection at 2.0×10^4 cells/well. Charcoal-absorbed (CA-) FBS was purchased from HyClone Laboratories, and delipidated CA-FBS was produced by treatment of CA-FBS serum with 3 g dry Cabosil resin/100 ml serum (Eastman Kodak, Rochester, NY) at 4 C for 24 h. Resin was removed by centrifugation and filtration.

HepG2 cells were transiently transfected by the calcium phosphate method with 20 μg total DNA in each precipitation in the ratio of 5 μg pRST7hER expression vector (or AR or PR expression vector), 10 μg LDLR-luciferase reporter, and 5 μg pRSV-β-galactosidase. After 20 min, 11 μl of precipitate containing 110 ng DNA was added to each well of a 96-well plate containing HepG2 cells. The precipitates were allowed to remain on cells for 6 h before being removed by aspiration. Cells were then washed with PBS and allowed to recover overnight in delipidated CA-FBS before drug was added the next morning in delipidated CA-FBS containing 1% dimethylsulfoxide. Cells were exposed to drug or hormone 40 h before lysis and determination of luciferase and β-galactosidase activity. Luciferase assays were performed on a fraction of cell lysate using a Dynatech 96-well luminometer. β-Galactosidase assays were performed on the remaining lysate using *O*-nitrophenyl-β-D-galactopyranoside as the enzyme substrate. To determine the normalized luciferase response, the number of luciferase relative light units for each well was divided by the β-galactosidase reaction rate for that well, where the β-galactosidase reaction rate is the final optical density × 10/reaction time (minutes). Each data point presented is the average of three replicate wells, except where noted as duplicate samples, with error bars representing the SD.

Results

LDLR transcription is activated by ER

To determine whether the ER may up-regulate LDLR transcription, a reporter plasmid containing the LDLR promoter driving expression of the firefly luciferase gene was transiently transfected into HepG2 cells. This reporter plasmid contained the natural human LDLR promoter region extending from −600 bp upstream to 53 bp downstream of the transcriptional start site and included the promoter elements required to observe sterol-regulated transcription (19). In the absence of cotransfected ER, induction of LDLR reporter transcription by 17β-estradiol was not observed (Fig. 1A). However, cotransfection of an expression vector for the human ER (hER) with the −600 LDLR reporter construct permitted a significant 6.5-fold LDLR transcriptional activation after incubation for 40 h with 17β-estradiol (Fig. 1A; $P < 0.006$ for 10 μM estrogen and $P < 0.025$ for 1 nM estrogen activation of LDLR with hER present), indicating that estrogen can activate LDLR transcription in an ER-dependent manner. The response of LDLR to estrogen was greater than the activation induced by the LDLR-elevating drug lovastatin (Fig. 1A), which was not affected by the presence of hER. The transcriptional response of LDLR to 17β-estradiol paralleled the response of a reporter construct driven by a canonical ERE to 17β-estradiol (Fig. 1B). The lack of estrogen response of either the ERE or the LDLR reporter in the absence of exogenously added ER indicates a lack of endogenous ER in this cell line. Taken together, these results indicate that 17β-estradiol up-regulates LDLR transcription in HepG2 cells in an ER-dependent manner.

LDLR transcriptional activation by estrogen occurs through sterol response elements

The LDLR promoter elements required to observe sterol regulation have been localized to binding sites for the transcription factors Sp1 and SREBP (19–21). A minimal LDLR promoter was constructed containing three copies of the LDLR Sp1- and SREBP-binding region, referred to as repeats 2 and 3, linked to the TATA region of the adenovirus E1b gene (Fig. 2A). As with the −600 LDLR reporter, 17β-estradiol had no effect on transcription of the minimal LDLR promoter in the absence of cotransfected hER (Fig. 2B), whereas lovastatin activated transcription 3.2-fold, and 25-hydroxycholesterol repressed LDLR transcription to 24% of

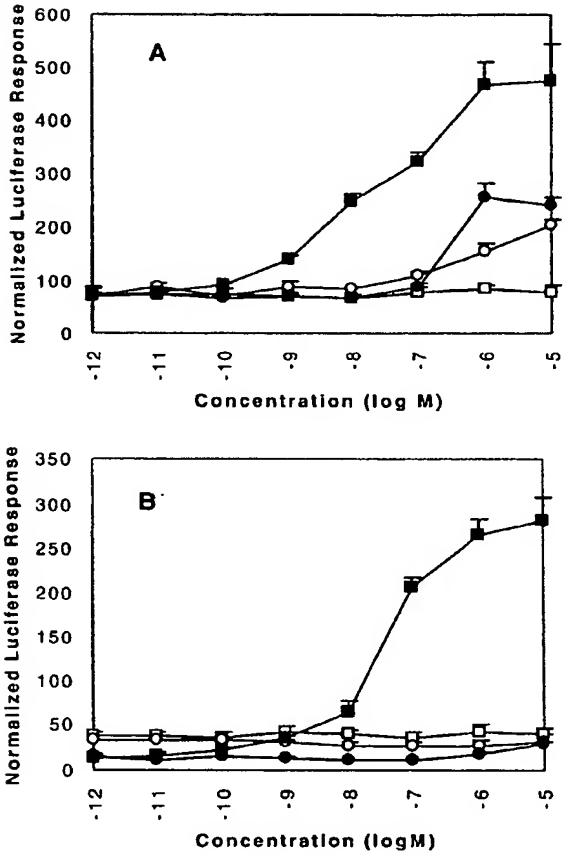

FIG. 1. ER activates LDLR transcription. A, The −600 LDLR promoter was transfected into HepG2 cells in the presence or absence of an expression vector for the hER. Cells were then exposed to progressively increasing concentrations of either 17β-estradiol or lovastatin for 40 h, followed by lysis and determination of luciferase and β-galactosidase activities. *Squares* represent luciferase activity in cells exposed to estrogen in either the presence (■) or absence (□) of transfected ER. *Circles* represent luciferase activity in cells exposed to lovastatin in either the presence (●) or absence (○) of transfected ER. B, Cells were transfected with ERE-tk-LUC reporter in the presence or absence of hER expression vector, followed by exposure of cells to either lovastatin or 17β-estradiol under the same conditions as those used to examine LDLR activation. The results shown are representative of those obtained in two separate experiments. *Squares* represent luciferase activity in cells exposed to estrogen in either the presence (■) or absence (□) of transfected ER. *Circles* represent luciferase activity in cells exposed to lovastatin in either the presence (●) or absence (○) of transfected ER.

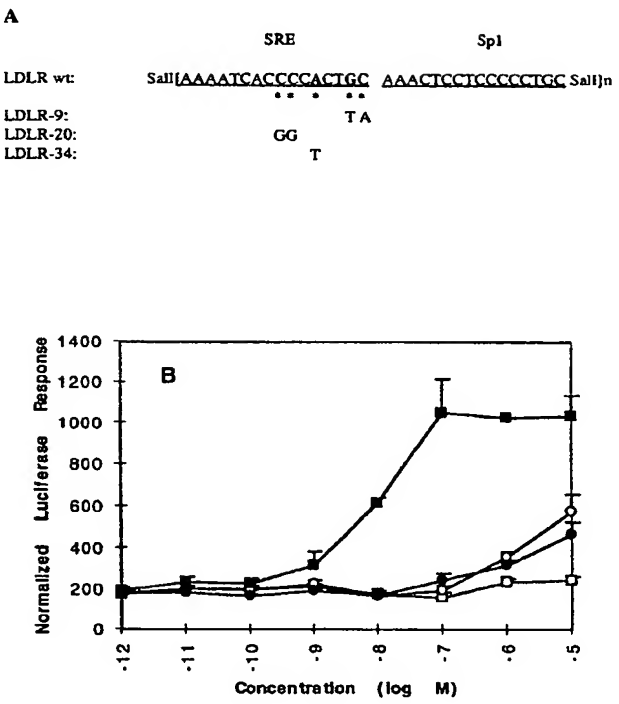

FIG. 2. ER activates a minimal LDLR promoter. A, The DNA sequence of the minimal LDLR promoter. B, The LDLR reporter containing multimerized binding sites for the transcription factors Sp1 and SREBP was transfected in HepG2 cells in the presence or absence of expression vector for hER, and then exposed to 17β-estradiol or lovastatin for 40 h. The results shown are representative of those obtained in 19 separate experiments. *Squares* represent luciferase activity in cells exposed to estrogen in either the presence (■) or absence (□) of transfected ER. *Circles* represent luciferase activity in cells exposed to lovastatin in either the presence (●) or absence (○) of transfected ER.

control levels (data not shown). The levels of induction by lovastatin and repression by 25-hydroxycholesterol are consistent with those reported previously for the regulation of LDLR in cotransfection experiments (19). Cotransfection of hER with the minimal LDLR promoter resulted in 5.5-fold induction by 17β-estradiol (Fig. 2B), but did not affect LDLR activation by lovastatin. The vector alone, containing the adenovirus E1b TATA region, was not activated by 17β-estradiol or hER (data not shown). Activation of the minimal LDLR promoter by ligand-bound ER occurred despite a lack of consensus EREs within the minimal LDLR promoter sequence.

To further examine the sequence requirements for regulation by the ER, mutations within the SREBP-binding site were constructed within the LDLR minimal promoter. Two different mutations within the SREBP-binding region of the promoter, LDLR-20 and LDLR-34 (see Fig. 2A for sequence), eliminated activation by either lovastatin or estrogen (Fig. 3). A mutation created outside of the SREBP-binding region, LDLR-9, had no affect on activation by either lovastatin or ER, suggesting that SREBP binding is required for activation of the LDLR promoter by estrogen. To examine the role of ER DNA binding in LDLR activation, two ER mutants deficient in DNA binding were examined for the ability to activate LDLR transcription (38, 39). Both of these mutants failed to activate LDLR (Fig. 4), suggesting that DNA binding is required for LDLR activation by ER. Although the LDLR promoter does not contain a consensus ERE, it mediates a response to estrogen as well as to sterol through the sterol response element.

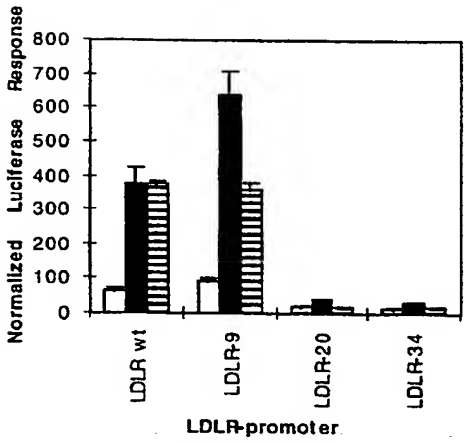

FIG. 3. Mutation of the LDLR SREBP-binding region prevents LDLR activation by ER. Oligos containing either the wild-type LDLR minimal promoter region or the mutations indicated in Fig. 2A were multimerized in two copies in luciferase reporter plasmids. These promoter mutants were cotransfected with expression vector for hER and exposed either to no drug (*open bars*) or to 100 nM 17β-estradiol (*black bars*) or 10 μM lovastatin (*striped bars*) for 40 h.

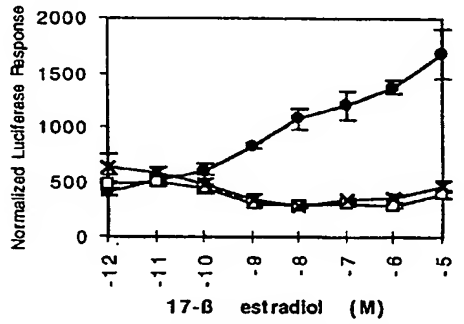

FIG. 4. Activation of the LDLR promoter requires an intact ER DNA-binding domain. HepG2 cells were cotransfected with the minimal LDLR promoter and expression vector for either wild-type hER or ER mutated at one of two different sites in the DNA-binding domain. Cells were then exposed to increasing concentrations of nM 17β-estradiol for 40 h before cell lysis and determination of luciferase activity. *Filled circles* represent activity with wild-type ER, *crosses* represent activity in the presence of ER-mutant 1, and *open squares* represent activity in the presence of ER-mutant 2.

ER antagonists block LDLR activation by estrogen

Several antagonists of the action of 17β-estradiol on the ER have been identified, including 4-hydroxytamoxifen, keoxifen, clomiphene, and ICI 164384 (40, 41). Tamoxifen has been reported to reduce atherosclerosis in animals (42) and to reduce fatal myocardial infarctions in women (43). We examined the activity of these ER modulators on ER-induced expression of LDLR reporters (Table 1). In the presence of 50 nM 17β-estradiol, the antagonists repressed estrogen-stimulated LDLR activation in a concentration-dependent fashion. Keoxifen antagonized LDLR induction by 17β-estradiol with an IC_{50} of 2.9 nM. The rank order of potencies of the antagonists were similar on LDLR and ERE promoters, with keoxifen being the most potent. Although keoxifen and tamoxifen can activate transcription on certain promoters (40, 41), we observed no activation of LDLR promoter-driven transcription by any of the ER antagonists in the HepG2 cell system (data not shown).

TABLE 1. Estrogen receptor antagonists repress ER-mediated LDLR activation

	ER/LDLR		ER/ERE-LUC	
	% Efficacy	IC_{50} (nM)	% Efficacy	IC_{50} (nM)
Keoxifene	83	2.9	84	1.5
ICI 182780	84	31	90	23
ICI 164384	85	370	85	101
Clomiphene	73	390	89	46
Tamoxifen	68	410	89	277
Toremifene	72	1100	87	485

Plasmid containing minimal LDLR promoter was cotransfected with hER expression vector. Cells were then exposed to increasing concentrations of ER antagonists from 10^{-12}–10^{-5} M in the presence or absence of 50 nM 17β-estradiol for 40 h. Efficacy is expressed as the percentage of maximal repression observed with antagonist compared to the response in the presence of 50 nM 17β-estradiol alone. The IC_{50} is the concentration of antagonist required to repress the response to 50% of the level induced by 50 nM 17β-estradiol alone. Efficacy and IC_{50} values for LDLR are shown from a representative experiment. Efficacy and IC_{50} for ER on the ERE-tk-LUC reporter are derived from an average of several experiments performed under similar conditions.

Androgenic sex steroids antagonize LDLR activation by ER

To test whether the activation of LDLR occurs with other sex steroid receptors, the PR and AR were each cotransfected with the LDLR promoter under the same conditions as those used to examine activation of LDLR by ER. PR did not activate LDLR in the presence of progesterone (Fig. 5A), whereas PR activated a reporter containing the MMTV enhancer region 48-fold (Fig. 5B). AR also failed to activate LDLR with the potent, metabolically stable AR agonist, R1881, whereas the MMTV enhancer was activated 73-fold under the same conditions (Fig. 5B). The activation of LDLR appeared specific to ER and was not a generalized transcriptional response to steroid hormone receptors.

Progestins are coadministered with estrogens in many hormone replacement regimens, however, and there is AR cross-reactivity of some currently used progestins. We determined whether activated AR or PR could alter estrogen-induced transcription of the LDLR reporter. Expression plasmids for AR or PR were cotransfected together with ER and the LDLR reporter constructs, and we measured the effects of various sex steroids. When ER alone was cotransfected with either an ERE-driven reporter or the LDLR reporter, progestins such as progesterone or MPA had no affect on transcription (Fig. 6A). The androgen R1881 displayed some activation of ER at this concentration, although it was at least 1000-fold more potent on AR than on ER. Estrogen-induced LDLR reporter transcription was also unaffected by progestins when LDLR was cotransfected with either ER alone or ER and PR together (Fig. 6B). When AR was cotransfected with ER and the LDLR promoter, however, the androgen

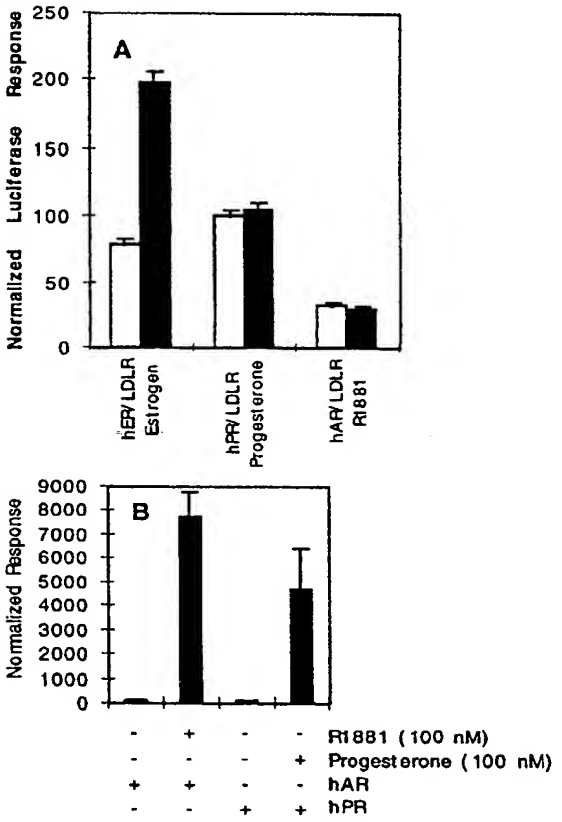

FIG. 5. Neither AR nor PR activates LDLR transcription. A, HepG2 cells were transiently transfected with the LDLR minimal promoter and expression vector for human ER, AR, or PR. Cells were then exposed to 100 nM 17β-estradiol (ER), R1881 (AR), or progesterone (PR) for 40 h before cell lysis and determination of luciferase and β-galactosidase activities. The results shown are representative of those obtained in two separate experiments. *White bars* represent reporter response in the absence of hormone, and *black bars* represent reporter activity in the presence of 100 nM of the indicated hormone B. HepG2 cells were transiently transfected with an MTV-enhancer-driven luciferase reporter and expression vector for either human AR or PR. Cells were then exposed to solvent alone, to 100 nM R1881 in duplicate with hAR, or to progesterone with hPR for 40 h before cell lysis and determination of luciferase and β-galactosidase activities.

R1881 strongly opposed LDLR activation by 17β-estradiol (Fig. 6C; $P < 0.005$ for repression by 1 nM R1881). Whereas progesterone did not affect LDLR transcription under any of the conditions tested, MPA repressed estrogen-induced LDLR reporter transcription by up to 50% when AR was present, consistent with its partial androgen agonist activity (Fig. 6C; $P < 0.0087$ for repression by 100 nM MPA). The down-regulation of LDLR by R1881 was not affected by the concentration of 17β-estradiol, indicating that androgens do not inhibit the action of 17β-estradiol by blocking binding to the ER (data not shown). The AR-dependent repression of LDLR by R1881 or MPA was relieved by the AR antagonist casodex, supporting the pharmacological specificity of the AR-mediated inhibition of LDLR activa-

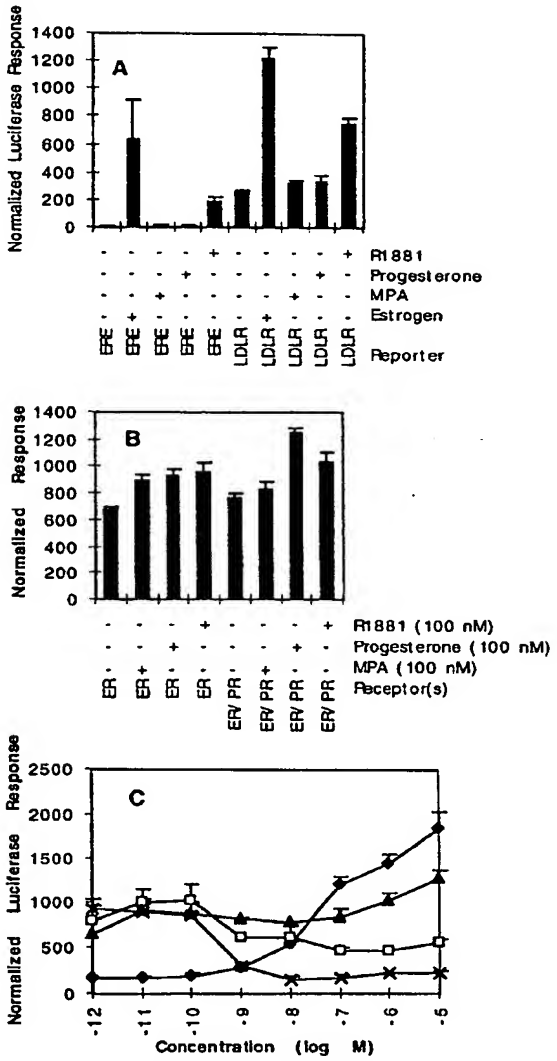

FIG. 6. The AR, but not the PR, opposes LDLR activation by ER. A, HepG2 cells were transiently transfected with LDLR reporter or ERE-tk-LUC reporter and expression vector for ER alone. Cells were then exposed to 100 nM of the indicated drug for 40 h before determination of luciferase activity. B, HepG2 cells were transiently transfected with LDLR reporter and either ER alone or ER in combination with PR. Cells were then exposed to 100 nM of the indicated drug for 40 h before determination of luciferase activity. C, HepG2 cells were transiently transfected with LDLR reporter and ER in combination with AR then exposed to increasing concentrations of 17β-estradiol alone or 30 nM 17β-estradiol together with increasing concentrations of progesterone, MPA, or R1881. Forty hours after the addition of drug, cells were lysed, and luciferase activity was determined. *Diamonds* represent the luciferase activity of cells exposed to increasing concentrations of estrogen, *open squares* represent the luciferase activity of cells exposed to 30 nM estrogen and increasing concentrations of MPA, *triangles* represent the luciferase activity of cells exposed to 30 nM estrogen and increasing concentrations of progesterone, and *crosses* represent the luciferase activity of cells exposed to 30 nM estrogen and increasing concentrations of R1881.

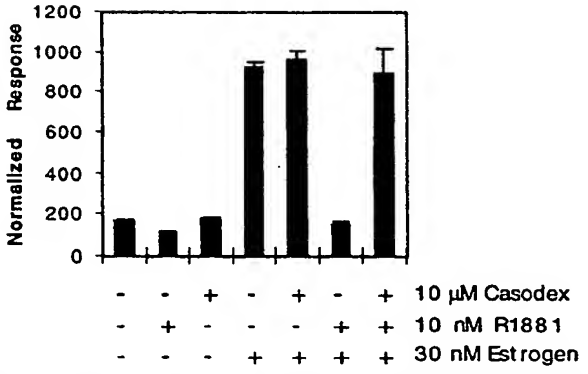

FIG. 7. LDLR repression is mediated in a pharmacologically specific manner by the AR. ER and AR were cotransfected with LDLR reporter, and the cells were exposed to combinations of 10 μM Casodex (Ligand Medicinal Chemistry, San Diego, CA), 30 nM 17β-estradiol, and 10 nM R1881. Forty hours after the addition of drug, cells were lysed, and luciferase activity was determined. The results shown are representative of those obtained in two separate experiments.

tion by ER (Fig. 7). These results indicate that the activation of LDLR by ER can be opposed by AR in the presence of androgenic steroids.

Discussion

There is considerable evidence that the cardioprotective effect of estrogen is mediated in part through lowering of plasma LDL cholesterol. Studies have demonstrated a significant increase in hepatic LDL-binding activity in response to estrogen treatment (24), but it has remained unclear whether LDLR is regulated by estrogen at the transcriptional level. We report here that the ER up-regulates LDLR transcription in transiently transfected HepG2 cells in a hormone-dependent manner, supporting the hypothesis that the reduction of plasma LDL by estrogen results from transcriptional up-regulation of LDLR.

The LDLR promoter element that mediates the estrogen response has been localized to the sterol regulatory region bound by the transcription factors Sp1 and SREBP (19–21). Cleavage of SREBP in response to intracellular levels of cholesterol is thought to be the mechanism by which cholesterol and lovastatin regulate LDLR transcription. This promoter region does not contain a consensus or near-consensus ERE or even a clear half-site through which ER could bind directly. Gel shift experiments with ER confirm a lack of ER-binding sites in this region,[1] further indicating that ER does not activate LDLR by directly binding to the LDLR promoter. SREBP binding does appear necessary for activation of LDLR by ER, however, suggesting that ER may activate the promoter indirectly, through transcriptional activation of SREBP or other factors that regulate LDLR. Curiously, although ER does not appear to bind the LDLR promoter, activation of the LDLR promoter requires an intact ER DNA-binding domain. LDLR activation by ER provides an additional example of transcriptional regulation by ER in the absence of a consensus ERE (38–41, 44).

In addition to estrogen, cardioprotective effects have been reported for ER antagonists such as tamoxifen. Women receiving tamoxifen as treatment for breast cancer displayed lowered plasma LDL (45–47). We did not observe activation of LDLR transcription by ER antagonists, including tamoxifen. Some of the cardioprotective actions of estrogen and other ER modulators are likely to occur through mechanisms other than transcriptional up-regulation of LDLR. Tamoxifen reduced experimental atherosclerosis in mice (42), but caused only a small reduction in LDL cholesterol and a large reduction in HDL cholesterol, suggesting that the plasma lipid alterations were not responsible for the decreased plaque formation observed. A mechanism that was suggested for the reduced plaque formation in mice was increased expression of transforming growth factor-β. Rudling *et al.* have found that 17α-ethinyl estradiol, but not tamoxifen, up-regulated LDLR expression in rat liver and that tamoxifen reduced HDL, but not LDL, in cholesterol-fed rats.[2] Inhibition of LDLR oxidation by tamoxifen has been suggested as one cardioprotective mechanism (47, 48), and alteration of lipid profiles in women taking tamoxifen may occur through altered secretion or exchange of lipoprotein particles. Although there are multiple mechanisms that are likely to be responsible for the cardioprotective actions of estrogen, cotransfection of ER with LDLR may provide a useful model to predict the capacity of ER modulators to lower plasma LDL cholesterol levels by increasing LDLR expression.

The cardioprotective benefits associated with estrogen do not appear to be associated with other sex steroids, including progestins and androgens. Furthermore, progestins attenuate the cardioprotective effects of estrogen (16, 27–29), particularly 19-nortestosterone-derived progestins. The opposition of estrogen's cardioprotection may be related to the androgenic activity of progestins (30–32). MPA, which has activity as an AR agonist, elevates LDL cholesterol and reduces the cardioprotection afforded by estrogen (16, 27–29). In concordance with the elevation of plasma LDL cholesterol by androgens or androgenic progestins in women, AR and androgenic steroids oppose the activation of LDLR by ER in these cotransfection experiments.

There are several potential explanations for the AR-mediated opposition of LDLR activation by ER. AR-mediated repression does not appear to be a general repression of transcription, since AR has no affect on LDLR in the absence of ER. Repression of AR-mediated activation by ER has been reported (49), but this does not distinguish the mechanism of interaction between the receptors. One potential mechanism is that AR contacts ER directly to block activation by ER. Another possibility is that AR prevents ER activation of LDLR by competing for a cofactor shared by ER and AR. Such a cofactor would apparently not be shared by PR, as PR did not repress LDLR. At present, none of the coactivators identified appear specific to AR and ER, but not to PR. An example of receptors negatively regulating each other by titration of a common cofactor is provided by the recent observation that retinoid receptors

[1] Allegretto, B., Ligand Pharmaceuticals, personal communication.

[2] Rudling, M., personal communication.

block activating protein-1 activity by competing for the CREB binding protein cofactor (50). CREB binding protein has been observed to interact with SREBP and to mediate SREBP transcriptional activity as well as that of the steroid hormone receptors, increasing the likelihood of such a mechanism (51).

Estrogen is believed to play a key role in maintaining cardiovascular health in women by several mechanisms, including lowering plasma LDL cholesterol levels. Although LDLR activation by estrogen does not account for all of the cardioprotective effects of estrogen, the cotransfection of ER with LDLR may provide an assay to study estrogen-mediated cardioprotection ascribed to lowering of plasma LDL cholesterol. The opposition by AR of LDLR activation by ER may also represent a useful model for characterization of the effects of sex steroids on plasma lipids during postmenopausal hormone replacement therapy. Future experiments will focus on the molecular mechanism by which the LDLR promoter responds to ER and AR to further clarify the complex interactions between sex steroids and cardiovascular health.

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Erratum

In the article "Regulation of apoptosis in uterine leiomyomata" by K. D. Burroughs, K. Kiguchi, S. R. Howe, R. Fuchs-Young, D. Trono, J. C. Barrett, and C. Walker (*Endocrinology* **138**: 3056–3064, 1997), Figure 4 appeared too dark when it was printed. The figure and its legend appear below. The printer regrets the error.

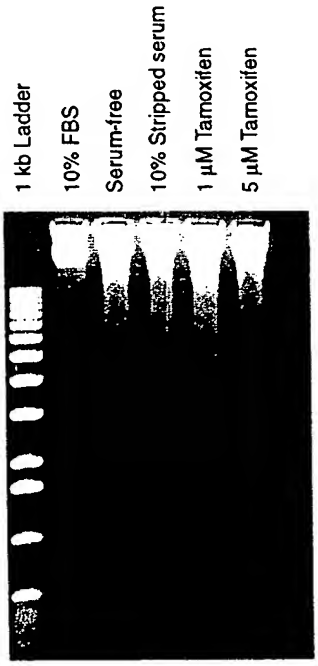

FIG. 4. DNA ladders. Genomic DNA isolated from ELT-3 cells grown in 10% FBS, serum-free medium, 10% stripped serum, 1 μM tamoxifen, and 5 μm tamoxifen in 10% stripped serum was electrophoresed on a 1% agarose gel. A distinct DNA ladder was present in cells grown in the absence of serum.